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Expression of human cytochrome P450 1A2 in *Escherichia coli*: a system for biotransformation and genotoxicity studies of chemical carcinogens

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In this study we describe the development of strain BMX100, a new *Escherichia coli* K12 tester strain, derived from MX100, a strain which was constructed for detection of mutagens and for mechanistic studies of chemical carcinogens. We demonstrate here that strain BMX100 can be used for stable expression of human CYP1A2 or human CYP1A2 fused to rat liver NADPH cytochrome P450 reductase. Mutagenicity of precarcinogens known to be bioactivated by CYP1A2, namely 2-aminoanthracene (2-AA), aflatoxin B1 (AFB1) and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), could be detected. The mutagenic activity of 2-AA using BMX100 expressing CYP1A2 alone and in combination with rat CYP reductase was respectively 10 and 20 times higher than in BMX100 with the standard metabolic activation system, rat liver S9 fraction. Furthermore, the mutagenicity of 2-AA could be nullified by α-naphthoflavone, a known inhibitor of CYP1A2. IQ responded equally in BMX100 expressing the CYP1A2–reductase fusion protein as compared with usage of rat liver S9 fraction. Rat liver S9 fraction was much more potent in generating a mutagenic response to AFB1 in BMX100 than in the strain expressing human CYP1A2 alone or CYP1A2 fused to rat reductase. The results described in this study demonstrate that this new *E.coli* strain can function as a human CYP1A2-competent prokaryotic mutagenicity test system and they seem to characterize BMX100 as a strain of interest for studies to identify individual human CYPs involved in bioactivation and bioinactivation reactions of putative genotoxins.

Introduction

Metabolism of chemical carcinogens plays a major role in the aetiology of cancer (Gonzalez and Gelboin, 1994). A better understanding of the properties of carcinogen metabolizing enzymes will thus aid in studies of human risk assessment. The majority of cell systems currently used for genotoxicity assays are not competent for metabolism of xenobiotics or they have lost this capacity (Miller and Miller, 1981; Langenbach *et al.*, 1992). These cell systems are therefore often combined with an exogenous metabolic activation system, usually a hepatic post-mitochondrial supernatant (S9). Although the combination of metabolic activation systems with these cell systems has contributed much to the success of genotoxicity assays, application of metabolic activation systems still has a number of drawbacks (Bridges and Hubbard, 1980; Langenbach *et al.*, 1992). Since metabolic activation occurs outside the target cell, the sensitivity for detection of highly reactive, short-lived metabolic intermediates may be limited. Furthermore, enzyme activities of these metabolic activation systems are highly variable and usually constitute varying concentrations of multiple (iso)enzymes, which make it difficult to determine the relative contribution of individual (iso)enzymes (Langenbach *et al.*, 1992).

In order to overcome these drawbacks novel metabolically competent cell systems for use in genotoxicity testing are being developed (Gonzalez and Gelboin, 1994; Rueff *et al.*, 1996). One approach to determine the contribution of specific mammalian (iso)enzymes in bio(in)activation of chemical carcinogens is by heterologous expression of mammalian cDNAs in the target cell (Gonzalez *et al.*, 1991; Langenbach *et al.*, 1992). Using this approach for prokaryotic genotoxicity assays a number of studies have been reported, e.g. for glutathione S-transferase (GST) (Simula *et al.*, 1993; Thier *et al.*, 1993), N-acetyltransferase (NAT) (Grant *et al.*, 1992), sulfotransferases (Glatt, 1997) and cytochrome P450 (CYP) (Josephy *et al.*, 1995).

Recently we reported on the construction of a new *Escherichia coli* K12 genotoxicity tester strain MX100, especially designed for the study of mechanistic aspects of genotoxic carcinogens (Kranendonk *et al.*, 1996). MX100 is sensitive to a variety of mutagens, including oxidative mutagens, which are monitored by back mutation to L-arginine prototrophy via base substitution mutations. Since this strain is derived from the well-known *E.coli* K12 laboratory strain AB1157, MX100 is a useful strain for development of specialized tester strains in metabolic studies of chemical carcinogens, notably by heterologous expression of specific mammalian drug metabolizing enzymes (Kranendonk *et al.*, 1996).

A prerequisite for the use of MX100 and other cell systems as a mammalian enzyme expressing cell system is that the cell system itself is characterized for endogenous biotransformation capabilities, as these could interfere with the bio(in)activation activities of the expressed enzyme and might lead to incorrect conclusions concerning the role of the expressed enzyme in bio(in)activation of a chemical. A comprehensive characterization of bioactivation and bioinactivation enzyme activities of strain MX100 has recently been performed (Kranendonk *et al.*, 1997). MX100 demonstrated significant metabolic activities of phase I enzymes (nitroreductase and DT-diaphorase), phase II enzymes (GSTs, NATs and UDP-glucuronyl transferases), phase III enzymes (cysteine conjugating β-lyase) and antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase and alkyl hydroperoxide reductase) and it contained appreciable levels of glutathione (GSH). Furthermore, a significant NADPH-flavodoxin:oxidoreductase activity was detected in MX100. It has been reported that this enzyme is able to support the activity of heterologously expressed

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mammalian CYPs (Jenkins and Waterman, 1994). CYP is considered to be the most important phase I biotransformation enzyme involved in bioactivation of chemical carcinogens (Gonzalez and Gelboin, 1994, Goeptar *et al.*, 1995).

We report here on the construction of a novel tester strain BMX100, a derivative of strain MX100, designed for stable heterologous expression of mammalian metabolic enzymes. We demonstrate here that BMX100 can actively express human CYP1A2 alone or as a fusion with rat liver NADPH CYP reductase and that it demonstrates sensitive mutagenic responses towards specific precarcinogens.

Materials and methods

Reagents and enzymes

4-Nitroquinoline-1-oxide (4NQO), L-arginine, aflatoxin B1 (AFB1), 2-aminoathracene (2-AA), α-naphthoflavone (α-NF), isopropylthiogalactoside (IPTG), ATP and thiamine were obtained from Sigma Chemical Co. (St Louis, MO). Ampicillin (sodium salt) was from Northumbria Biologicals (Northumberland, UK). Bacto agar, bacto tryptone, bacto yeast extract and bacto peptone were from Difco (Detroit, MI). The antibiotic kanamycin sulphate was obtained from Boeringher-Mannheim (Mannheim, Germany). Restriction enzymes *Bam*HI, *Pst*I and *Acc*I were obtained from Stratagene (La Jolla, CA) and T4 DNA ligase from Promega (Madison, WI). Plasmid pACYC177 was obtained from New England Biolabs (Beverly, MA). 2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) was a generous gift of Dr Grivas (Swedish University of Agriculture Sciences, Uppsala, Sweden).

Strains and plasmids

A new mutator plasmid was constructed to circumvent plasmid incompatibility. The *mucAB* operon was excised from plasmid pGW270 (Langer *et al.*, 1981) as a 2.5 kb *Pst*I–*Acc*I fragment and subcloned into pUC18, previously digested with *Pst*I and *Acc*I. Plasmid DNA was transfected into DH5α cells by the $CaCl_2$ method (Sambrook *et al.*, 1989). A clone was selected and plasmid DNA of this new plasmid (pHCM) was isolated. Subsequently, the *mucAB* operon was excised from pHCM as a 3.1 kb *Bam*HI–*Pst*I fragment and cloned in plasmid pACYC177 (Chang,A.C.Y and Cohen, 1978), previously digested with *Bam*HI and *Pst*I. Plasmid DNA was transfected into XL1-blue Epicurian Coli competent cells (Stratagene) by the procedure described by the manufacturer. The new plasmid (pLCM) was isolated and transfected into strain FP401, the mother strain of the *E.coli* K12 tester strain MX100 (Kranendonk *et al.*, 1996), by the $CaCl_2$ method and selected for kanamycin-resistant growth. The new strain was designated BMX100.

Plasmids pCWori+, containing the modified human *CYP1A2* sequence (Fisher *et al.*, 1992), and pCWori+, containing the modified human *CYP1A2* sequence fused to the rat NADPH CYP reductase sequence (Shet *et al.*, 1995), were transfected into BMX100 by the $CaCl_2$ method, resulting in strains BMX100/h1A2 and BMX100/h1A2-rRed respectively. Both plasmids were a generous gift of Dr Fisher and Prof. Estabrook (Southwestern Medical Center, University of Texas, Dallas, TX).

Analysis of CYP expression

Expression of CYP in bacteria was analysed spectrophotometrically (difference spectrum, reduced–carbon monoxide versus reduced) as previously described, with minor modifications (Rutten *et al.*, 1987) Cultures of BMX100/h1A2 and BMX100/h1A2-rRed, grown as described below, were centrifuged at 2600 *g* for 15 min at 4°C. Bacterial pellets were resuspended in the same volume of TN buffer (50 mM Tris, 0.85% NaCl, pH 7.5). Spectra were recorded using a Pye Unicam SP8-100 UV/VIS spectrophotometer

Mutagenicity assays

Strains BMX100/h1A2 and BMX100/h1A2-rRed were cultured as previously described with minor modifications (Fisher *et al.*, 1992) Bacteria were grown for 18 h in TB medium supplemented with peptone (2 g/l), ampicillin (50 μg/ml), kanamycin (15 μg/ml), thiamine (1 μg/ml), a mixture of trace elements (0.4 ml/l) (Bauer and Shiloach, 1974) and IPTG (1 mM) as appropriate at 29°C. Hepatic post-mitochondrial supernatant (S9) of male Wistar rats, induced with Aroclor 1254, was prepared as previously described (Maron and Ames, 1983) and applied in the standardized 10% mix (Maron and Ames, 1983) in the mutagenicity assays

The mutagenicity assays with strains BMX100/h1A2 and BMX100/h1A2-rRed were performed using the liquid preincubation assay technique as described by Maron and Ames (1983), with minor modifications Preincubation was performed for 45 min in an orbital shaker at 37°C, before plating. Incubation buffer contained 10 mM glucose. The inhibition study with α-naphthoflavone (α-NF) was performed with a 15 min incubation at 37°C with bacteria and inhibitor in buffer, prior to addition of the carcinogen.

Experiments were performed at least in triplicate. Revertant colonies were counted after the normal 48 h incubation at 37°C. Mutagenic activities (in revertants/nmol carcinogen) were determined from the slope as the least square line of the linear portion of the dose–response curve.

Results

The *E.coli* K12 tester strain MX100 has been developed for detection of mutagens and characterized as a strain of interest for mechanistic studies of chemical carcinogens (Kranendonk *et al.*, 1996; 1997). No CYP activity could be detected in strain MX100. This strain could therefore be applied as a heterologous expression system for CYPs. When plasmid pCWori+ containing the modified cDNA of human CYP1A2 (pCWori/h1A2) (Fisher *et al.*, 1992) was introduced into MX100 we found low and unstable expression of CYP and no detectable bioactivation activity for AFB1 and 2-AA, two compounds used as model genotoxins (data not shown). Strain MX100 contains the SOS mutagenesis plasmid pKR11, which is maintained at low copy number (Kranendonk *et al.*, 1994a, 1996). This plasmid is a deletion derivative of the standard mutator plasmid pKM101 (Langer *et al.*, 1981), which contains a ColE1-type replicon (Langer *et al.*, 1985). Plasmid pCWori+, a derivative of plasmid pHSe5, is a pBR322-derived plasmid and thus contains the pMB1 origin of replication (Browner *et al.*, 1991; Fisher *et al.*, 1992). High plasmid instability occurs when both plasmids are transferred to the same bacterial cell, a phenomenon known as plasmid incompatibility (Sambrook *et al.*, 1989), which was verified in MX100 when transformed with pCWori+.

Strain BMX100

The relevant sequences of plasmid pKR11 necessary for mutagenicity testing, the *muc*AB operon (Kranendonk *et al.*, 1994a), were cloned in pACYC177 (Chang,A.C.Y. and Cohen, 1978), to circumvent plasmid incompatibility of pKR11 and pCWori+ in MX100. pACYC177 is maintained at low copy number and contains replicon p15A, a replicon compatible with the ColE1 origin (Sambrook *et al.*, 1989). The newly constructed plasmid (pLCM) was introduced into the mother strain of MX100, FP401, resulting in strain BMX100. The efficiency of mutagen detection of strain BMX100 was checked, relative to strain MX100, with three diagnostic mutagens, namely 4NQO, benzo[*a*]pyrene (B[a]P) and AFB1, the last mutagens with the rat liver S9 fraction (see Figure 1). Results obtained with these carcinogens demonstrated that BMX100 responds similarly to MX100. Plasmid pCWori+, containing the modified cDNA of human CYP1A2, was subsequently introduced into strain BMX100, designated BMX100/h1A2.

Expression of human CYP1A2 in strain BMX100

Expression of human CYP1A2 in strain BMX100 was detected by optical difference spectrophotometry. When expression in strain BMX100/h1A2 was induced with 1 mM IPTG a CYP difference spectrum could be recorded, as illustrated in Figure 2a. The typical 449 nm absorbance maximum of the difference spectrum of CYP1A2 is present, as demonstrated previously in *E.coli* (Fisher *et al.*, 1992). Without IPTG strain BMX100/h1A2 demonstrated spectra as shown in Figure 2b, with absorbance minima at 444 and 430 nm and an absorbance maximum at 416 nm, which are characteristic for the bacterial respiratory cytochromes d and o (Porter and Larson, 1991).

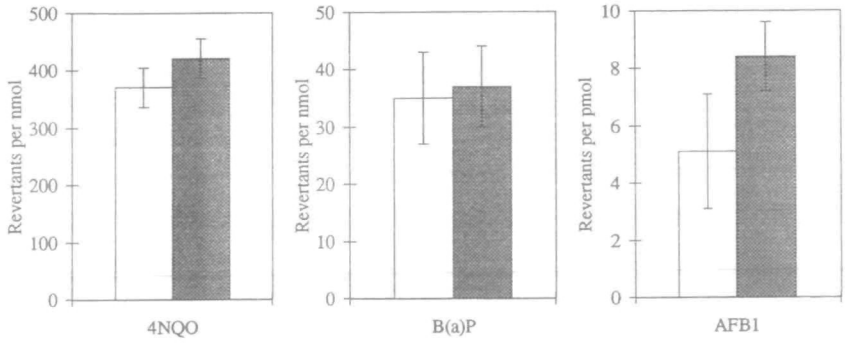

Fig. 1. Histograms of the mutagenic activity of 4NQO, B[a]P and AFB1 in strains MX100 (open blocks) and BMX100 (hatched blocks). B[a]P and AFB1 were tested with the standard 10% rat liver S9 mix. Bars, standard deviation.

Mutagenicity of 2-aminoanthracene in strains BMX100/h1A2 and BMX100/h1A2-rRed

When strain BMX100/h1A2, expressing CYP1A2, was used in a mutagenicity assay 2-AA showed a dose-dependent increase in mutagenicity (see Figure 3) with a mutagenic activity (revertants/nmol) 10 times higher than obtained with the traditional rat liver S9 fraction (see inset of Figure 3 and Table I). Another expression vector was subsequently introduced into strain BMX100, namely pCWori+, containing the modified cDNA of human CYP1A2 fused to cDNA of rat liver NADPH CYP reductase (pCWori/h1A2-rRed) (Shet *et al.*, 1995), resulting in strain BMX100/h1A2-rRed. The level of CYP expression in this strain was approximately one third of that measured for BMX100/h1A2. 2-AA demonstrated a 20-fold increase in mutagenic activity in strain BMX100/h1A2-rRed as compared with rat liver S9 (Figure 3 and Table I). No mutagenicity could be detected for 2-AA in strain BMX100/h1A2 without induction, as shown in Figure 3.

The mutagenicity of 2-AA in the induced strain BMX100/h1A2-rRed could be inhibited to background levels by α-naphthoflavone, a specific inhibitor of the CYP1A family (Chang,T.K.H. *et al.*, 1994), at a dose of 1 nmol/plate (Figure 4).

Mutagenicity of AFB1 and IQ in strains BMX100/1A2 and BMX100/h1A2-rRed

We also tested AFB1 in strain BMX100/h1A2 (Table I) as well as AFB1 and IQ in strain BMX100/h1A2-rRed (Figures 5 and 6 respectively). AFB1 demonstrated a mutagenic activity in strain BMX100/1A2 (Table I) which was increased by a factor of ~5 in strain BMX100/h1A2-rRed. (see Figure 5 and Table I). The mutagenic activity of AFB1 was substantially higher in BMX100 using the rat liver S9 fraction than in BMX100 expressing human CYP1A2 or in BMX100 expressing CYP1A2–reductase fusion protein (see Figure 5 and Table I). AFB1 showed no mutagenicity in BMX100 when CYP expression was not induced, as shown in Figure 5.

IQ was only tested in strain BMX100/h1A2-rRed and showed a mutagenicity similar to that obtained with the rat liver S9 fraction (Figure 6 and Table I). As previously shown for other carcinogens, no mutagenicity was detected for IQ when CYP expression was not induced in BMX100/h1A2-rRed.

Discussion

A number of approaches are available to assess the role of drug and xenobiotic metabolizing enzymes in activation and/or inactivation of chemicals that might act as potential carcinogens (Miles and Wolf, 1991). Heterologous expression of human enzymes in a cell system used for mutagenicity testing has the advantage that metabolites are generated inside the target cell and thus enable study of the role of a single gene product in bio(in)activation of chemicals.

CYPs mediate major routes in bioactivation of chemical carcinogens (Guengerich, 1992; Gonzalez and Gelboin, 1994). In this study we report on the development of a new *E.coli* tester strain, BMX100, which is able to express active human CYP1A2, alone or fused to rat liver NADPH CYP reductase. With these strains we were able to detect bioactivation of 2-AA, AFB1 and IQ. These three precarcinogens are known to become more genotoxic following oxidative metabolism catalysed by CYP1A2 (Gonzalez and Korzekwa, 1994).

2-AA demonstrated a 10-fold higher mutagenic activity in strain BMX100 expressing human CYP1A2 than in BMX100 with the standard rat liver S9 fraction (Table I). This mutagenic activity was increased ~2-fold when tested in BMX100 expressing human CYP1A2 fused to rat liver NADPH CYP reductase. Furthermore, the mutagenicity of 2-AA could be nullified by α-naphthoflavone, a known inhibitor of the CYP1A family (Chang,T.K.H. *et al.*, 1994), confirming the bioactivation capacity of human CYP1A2 expressed in BMX100. Aromatic amines, like 2-AA, are activated by *N*-hydroxylation and subsequent *O*-acetylation mediated by CYP1A2 and NAT respectively (Gonzalez and Gelboin, 1994). As previously shown, strain MX100 contains a bacterial NAT activity (Kranendonk *et al.*, 1997). This activity is probably responsible for the observed mutagenicity of 2-AA in BMX100/h1A2 and BMX100/h1A2-rRed.

The potent hepatocarcinogen AFB1 demonstrated a mutagenic response when tested in strain BMX100, expressing human CYP1A2 alone, which is increased ~5-fold when the strain contained CYP1A2 fused to rat NADPH CYP reductase (Table I). Both these mutagenic activities were substantially lower than the activity in BMX100 supported by rat liver S9 fraction. AFB1 is activated by CYP to the ultimate carcinogen, AFB1-8,9-epoxide (Eaton and Gallagher, 1994). Multiple forms of CYP seem to be involved in metabolism of AFB1 and at least five isoforms are able to activate this carcinogen (Aoyama *et al.*, 1990). CYP1A2 and 3A4 are considered to be the more important isoforms (Crespi *et al.*, 1991; Gallagher *et al.*, 1994). Furthermore, it has been previously shown that there is a marked species difference in bioactivation of AFB1 between rat and human (Ramsdell and Eaton, 1990). Rat liver microsomes are far more efficient in generating AFB1-8,9-epoxide than human liver microsomes. This fact could, in part, explain the higher mutagenic activity observed with rat liver S9 in BMX100 as well as the fact that rat liver S9 contains

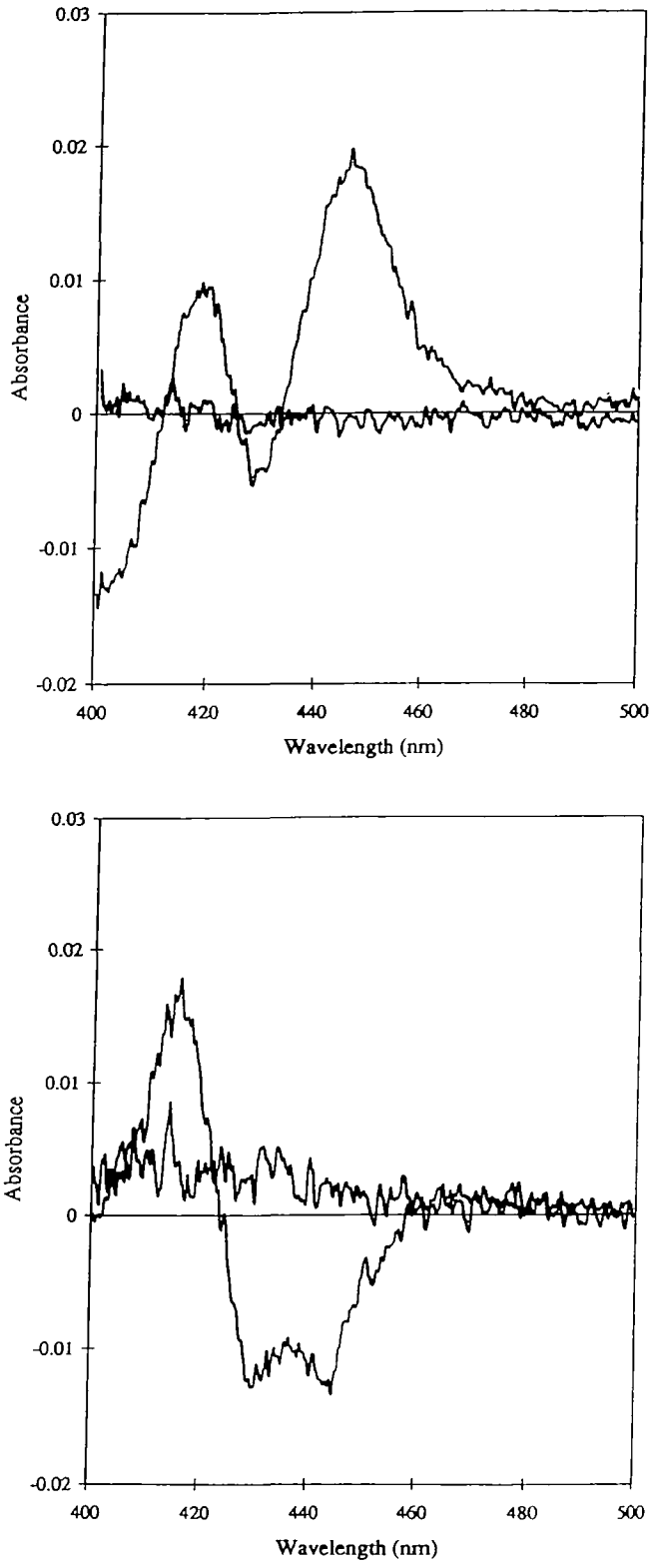

Fig. 2. Fe^{2+}–CO versus Fe^{2+} difference spectra of bacteria of strain BMX100/h1A2 in which expression of human CYP1A2 was induced (upper panel) or not induced (lower panel). The spectra shown in the upper panel indicate expression of 209 nmol P450 per liter of culture.

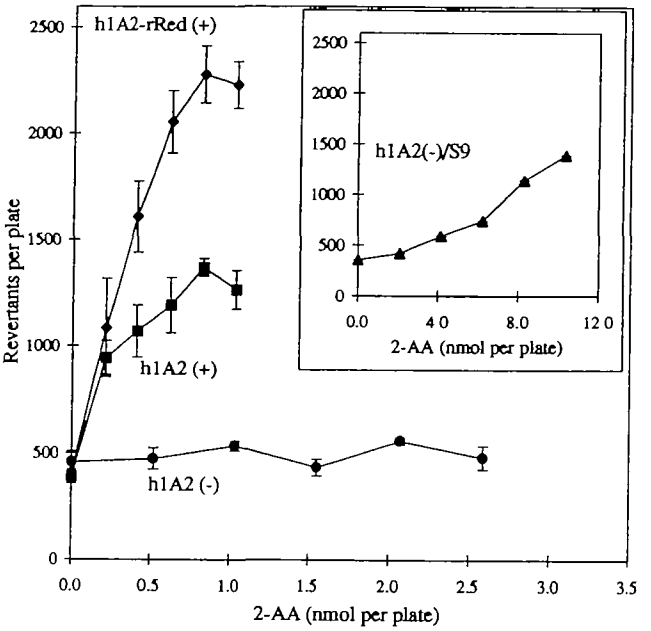

Fig. 3. Mutagenicity of 2-AA in strain BMX100 with the two expression vectors for human CYP1A2. h1A2, strain BMX100 containing the expression vector with cDNA of human CYP1A2; h1A2-rRed, strain BMX100 containing the expression vector with cDNA of human CYP1A2 fused to cDNA of rat liver NADPH CYP reductase; +, induction of expression with 1 mM IPTG; –, no induction of expression, points, mean of at least triplicate determinations, bars, standard deviation. For inset S9, standard (10%) rat liver S9 mix.

many other CYP isoforms which are involved in bioactivation of AFB1.

The heterocyclic aromatic amine IQ, a potent food carcinogen (Stavric, 1994), was mutagenic in strain BMX100 expressing human CYP1A2 fused to rat CYP reductase. This mutagenicity in BMX100/h1A2-rRed was similar to that found for BMX100 supported by the rat liver S9 fraction. IQ is metabolized by CYP1A2 to its hydroxylamine, but ester formation, in which acetyltransferase and sulfotransferase are thought to be of importance, is believed to be necessary for production of the ultimate carcinogen (Kadlubar *et al.*, 1992). As mentioned before, acetyltransferase activity was demonstrated in strain MX100, but no sulfotransferase activity could be detected (Kranendonk *et al.*, 1997). This seems to imply a role of bacterial NAT in the bioactivation of IQ described in this study.

IQ was ~10-fold less mutagenic in strain BMX100/h1A2-rRed than 2-AA (Table I). Bioactivation of 2-AA and IQ has previously been described in the *Salmonella typhimurium* Umu-test with use of an exogenous metabolic system, derived from *E.coli* membranes containing human CYP1A2 (Shimada *et al.*, 1994). The *Salmonella* tester strain used also contained an increased level of bacterial NAT. 2-AA and IQ demonstrated considerable but similar *umu*-inducing capacities in this test. The difference in mutagenicity for IQ in the two systems could be due to limiting NAT activity in BMX100, although this is not reflected in our results for 2-AA. Furthermore, bioactivated IQ intermediates could have similar *umu*-inducing capacities but be less efficient in inducing arginine reversion in BMX100 as compared with bioactivated 2-AA.

Strain BMX100/h1A2 showed a 3-fold higher CYP1A2 expression level as compared with strain BMX100/h1A2-rRed. Mutagenic activities were therefore normalized (in revertants/nmol.pmol CYP) for the plated (expressed) amount of CYP, to correctly compare the two strains for bioactivation (Table I). 2-AA was approximately seven times more effectively bioactivated when using the system containing human CYP1A2

Table I. Mutagenic activities of three carcinogens in strain BMX100, expressing human CYP1A2, human CYP1A2 fused to rat cytochrome P450 reductase or with rat liver S9 fraction

Carcinogen	BMX100/h1A2		BMX100/h1A2-rRed		BMX100 + rat liver S9 mix
	rev/nmol	rev/nmol.pmol P450	rev/nmol	rev/nmol.pmol P450	rev/nmol
2-AA	1066 ± 227	23 ± 5	2277 ± 235	166 ± 17	104 ± 13[a]
AFB1	203 ± 18	5 ± 1	958 ± 106	70 ± 8	9751 ± 623[b]
IQ	ND	ND	202 ± 15	15 ± 1	278 ± 23[b]

Mutagenic activities are expressed as revertants/nmol carcinogen ± SD and normalized for the plated amount of cytochrome P450 as revertants/nmol carcinogen/pmol P450 ± SD.
h1A2, expression vector with cDNA of human P450 1A2; h1A2-rRed, expression vector with cDNA of human P450 1A2 fused to cDNA of rat liver NADPH P450 reductase; S9, standard (10%) rat liver S9 mix.
[a]Determined with strain BMX100/h1A2 without induction of expression.
[b]Determined with strain BMX100/h1A2-rRed without induction of expression.
ND, not determined.

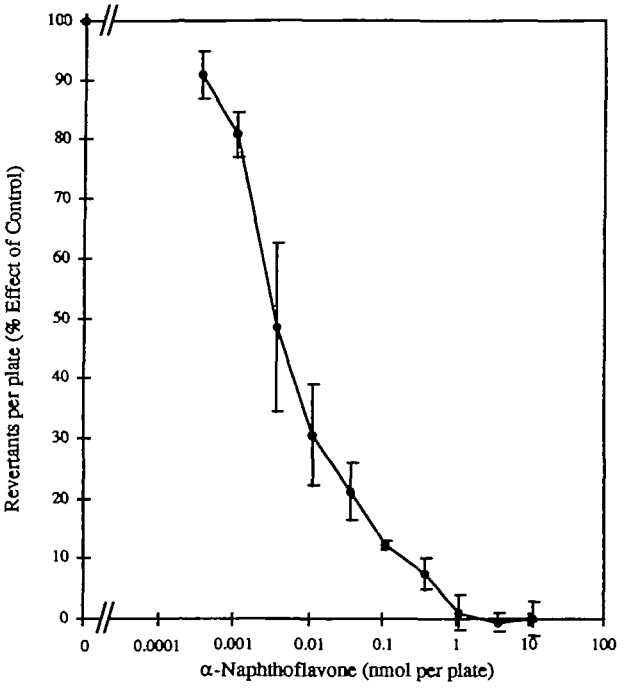

Fig. 4. α-Naphthoflavone inhibition of 2-AA mutagenicity in strain BMX100/h1A2-rRed with induction. Data are represented as a percentage of the control response (0.41 nmol 2-AA, 1519 revertants/plate). Points, mean of at least triplicate determinations; bars, standard deviation.

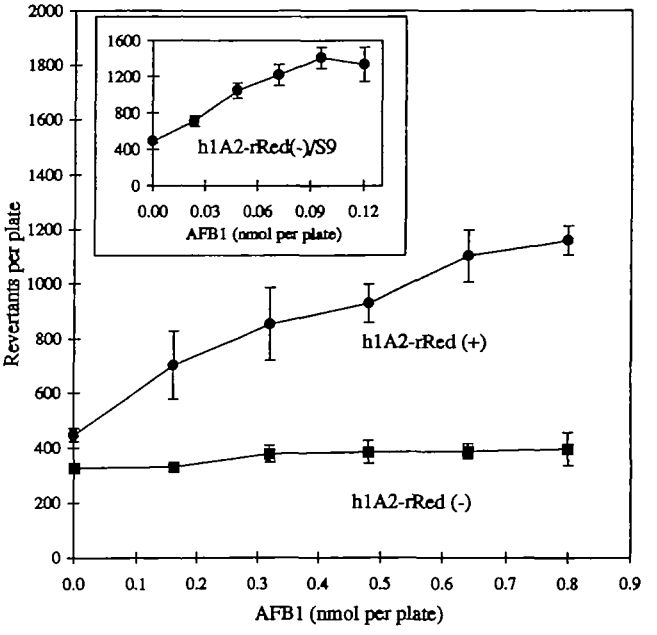

Fig. 5. Mutagenicity of AFB1 in strain BMX100 containing the expression vector with cDNA of human CYP1A2 fused to cDNA of rat liver NADPH P450 reductase. For an explanation of symbols used see Figure 2. Points, mean of at least triplicate determinations; bars, standard deviation.

fused to rat CYP reductase as compared with the system containing CYP1A2 alone. For AFB1 this increase was ~14 times. The difference in increase is probably due to the fact that AFB1 is bioactivated in a single CYP1A2-mediated reaction as compared with 2-AA, which, besides the CYP1A2-mediated step, needs a subsequent phase II reaction to become mutagenic, as described above.

We have previously reported on the detection of NADPH flavodoxin reductase in strain MX100 (Kranendonk *et al.*, 1997), a bacterial reductase which has been shown to be able to support mammalian CYP activity, albeit at a very slow rate (Jenkins and Waterman, 1994). This enzyme is probably responsible for the observed activation activities of human CYP1A2 in strain BMX100 when expressed without rat NADPH CYP reductase. The mutagenic activity of 2-AA in BMX100 expressing CYP1A2 alone and in combination with rat CYP reductase was respectively 10 and 20 times higher than BMX100 with the rat liver S9 fraction (Table I). To our knowledge this is the first time that mutagenicity is more effectively detected in a CYP-competent prokaryotic test system relative to using the rat liver S9 fraction with a non-competent prokaryotic system. These results confirm the notion that the usage of metabolically competent test systems can be more efficient in detection of mutagens relative to usage of exogenous metabolic systems, due to the extracellular generation of reactive metabolites.

In the last decade a number of heterologous expression systems, e.g. in bacteria, yeast, mammalian and insect cells, have been developed for mammalian CYPs and used for various types of studies (for reviews see Gonzalez and Korzekwa, 1994; Waterman, 1994). Some of these systems have been used to design CYP competent mutagenicity test systems, predominantly in mammalian cells (Gonzalez and Gelboin, 1994). The highest expression levels of mammalian CYPs were obtained in bacteria, namely in *E.coli* (Gonzalez and Korzekwa, 1994). Specific *E.coli* expression vectors were used and minor N-terminal modifications of the CYPs had to be made to optimize expression in *E.coli*, as was the case for the vectors

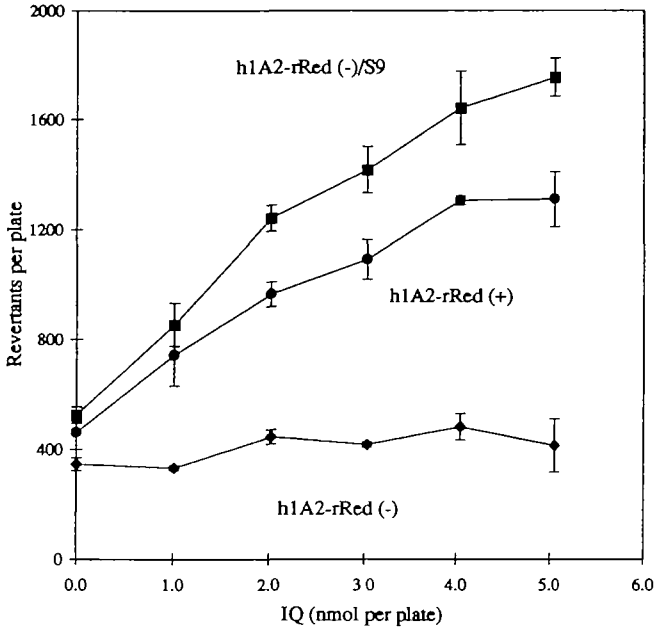

Fig. 6. Mutagenicity of IQ in strain BMX100 containing the expression vector with cDNA of human CYP1A2 fused to cDNA of rat liver NADPH P450 reductase. For an explanation of symbols used see Figure 2 Points, mean of at least triplicate determinations; bars, standard deviation

used in this study. The majority of these expression vectors are based on *LacI* repression, by which controllable expression of CYP can be obtained in *E.coli*. Due to the medium/high copy number of these plasmids, it is necessary that these vectors are introduced in a strain with a high *LacI* background (like strains JM105 and DH5α) or that the vector itself encodes for highly expressed *E.coli LacI*^{q} (such as the pCWori+ vector). As pointed out by us previously (Kranendonk *et al.*, 1994b), an *E.coli* tester strain seems to be more appropriate for heterologous expression with the above described *E.coli* vectors, as *S.typhimurium* lacks the *lac* operon and expression from these vectors was optimized for use in *E.coli*. Repression, and therefore controllability, of expression can be lost when mammalian metabolic enzymes are introduced into *S.typhimurium* tester strains with this type of *E.coli* vector. This has been shown for example for human GST (Simula *et al.*, 1993; Thier *et al.*, 1993; Oda *et al.*, 1996), human NAT (Grant *et al.*, 1992) and human CYP (Josephy *et al.*, 1995). Complete repression of expression of human CYP1A2 in strains BMX100/h1A2 and BMX100/h1A2-rREd seems to occur correctly, as no mutagenicity is observed for 2-AA, AFB1 and IQ and no CYP could be detected by spectrophotometric methods without induction. These results corroborate a previous study in which it was demonstrated that MX100 is devoid of CYP activities (Kranendonk *et al.*, 1997).

In conclusion, we have demonstrated here that strain BMX100 can be used for controllable heterologous expression of human CYP1A2 and can function as a human CYP1A2-competent prokaryotic mutagenicity test system in which bioactivation can be detected for precarcinogens known to be activated by CYP1A2. MX100, the mother strain of BMX100, has recently been comprehensively characterized for >20 endogenous biotransformation capabilities, so as to avoid incorrect conclusions concerning the role of heterologous expressed enzymes as well as to determine bacterial enzymes and factors which could function as accessory enzymes, such as NADPH flavodoxin reductase (Kranendonk *et al.*, 1997). In a previous study the activities of the major phase II enzymes were determined. Some of these activities are of importance for mutagenicity of chemical carcinogens in BMX100 as described here for 2-AA and IQ. However, precarcinogens other than 2-AA and IQ could require different phase II activities for bioactivation. Currently further characterization of strain BMX100 expressing human CYP1A2 is being performed with premutagens known to be bioactivated by CYP but by others then 1A2.

Since most research in this area is ultimately directed towards risk assessment of human exposure, there is an inherent advantage to using human enzymes. Strain BMX100 is apparently appropriate for expression of individual human CYP enzymes involved in bioactivation and bioinactivation reactions. At the moment other human CYP isoforms are being expressed in strain BMX100 and tested with different classes of precarcinogens.

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